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In Vitro Determination of Prebiotic Properties of Oligosaccharides Derived from an Orange Juice Manufacturing By-Product Stream

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Fermentation properties of oligosaccharides derived from orange peel pectin were assessed in mixed fecal bacterial culture. The orange peel oligosaccharide fraction contained glucose in addition to rhamnogalacturonan and xylogalacturonan pectic oligosaccharides. Twenty-four-hour, temperature- and pH-controlled, stirred anaerobic fecal batch cultures were used to determine the effects that oligosaccharides derived from orange products had on the composition of the fecal microbiota. The effects were measured through fluorescent in situ hybridization to determine changes in bacterial populations, fermentation end products were analyzed by high-performance liquid chromatography to assess short-chain fatty acid concentrations, and subsequently, a prebiotic index (PI) was determined. Pectic oligosaccharides (POS) were able to increase the bifidobacterial and *Eubacterium rectale* numbers, albeit resulting in a lower prebiotic index than that from fructo-oligosaccharide metabolism. Orange albedo maintained the growth of most bacterial populations and gave a PI similar to that of soluble starch. Fermentation of POS resulted in an increase in the *Eubacterium rectale* numbers and concomitantly increased butyrate production. In conclusion, this study has shown that POS can have a beneficial effect on the fecal microflora; however, a classical prebiotic effect was not found. An increase in the *Eubacterium rectale* population was found, and butyrate levels increased, which is of potential benefit to the host.

The concept of modulating gut health through diet is not new and dates back to at least the beginning of the 20th century. However, it is only recently that sound scientific rationales have been proposed and investigated. Three microflora modulation tools have emerged (40): the addition of exogenous living microorganisms to foods (i.e., probiotics), the selective stimulation of the growth and activity of beneficial microorganisms indigenous to the gut (i.e., prebiotics), and a combination of both approaches (i.e., synbiotics). All three approaches attempt to increase the numbers of bacteria seen as positive for human gastrointestinal health, usually the lactobacilli and bifidobacteria. Prebiotics have been defined as "non-digestible food ingredients that selectively stimulate a limited number of bacteria in the colon, to improve host health" (12). Bifidobacteria and lactobacilli are known to directly inhibit the growth of pathogenic bacteria, such as certain species of clostridia (e.g., *Clostridium difficile* and *C. perfringens*) and pathogenic *Enterobacteriaceae*, through the production of short-chain fatty acids, lowering of colonic pH, production of antimicrobial compounds, and/or competition for growth substrates and adhesion sites (13, 23, 27). The bifidogenic nature of a range of prebiotics has now been confirmed in vivo in human feeding studies (11, 37). These prebiotics include inulin, fructo-oligosaccharides (FOS), galacto-oligosaccharides, and lactulose (42, 43, 25, 44).

The aim of the present work was to assess the prebiotic properties of oligosaccharides extracted from the peel by-product of orange juice manufacturing. Citrus residue remaining after juice extraction represents approximately half of the fruit wet mass, and approximately half of this residue is peel (2). More than 2,000,000 tons of citrus pulp pellets are produced annually for cattle feed from citrus processing residue, which is a low-valued product. Pectin, a higher-valued product, is manufactured from citrus peel, but the market demand for pectin is low compared to the potential pectin that could be manufactured from the worldwide supply of citrus-processing residue.

Pectic oligosaccharides (POS) were manufactured from commercial pectin in an enzyme membrane reactor (30) and then evaluated for their prebiotic properties (29). These pectic oligosaccharides had a low prebiotic potential compared to FOS, although they were more selectively fermented than were the parent pectins (29). Pectic oligosaccharides also protected colonocytes against *Escherichia coli* verocytotoxins (32) and stimulated apoptosis in human colonic adenocarcinoma cells (31).

Here, we aimed to find a more cost-effective way to produce prebiotic pectic oligosaccharides directly from orange peel albedo (OA). Fructo-oligosaccharides, the most extensively studied prebiotics and the current market leader, were chosen as a positive control for in vitro fecal fermentation studies, while starch, a nonprebiotic carbohydrate, was used as a nonselective control.

MATERIALS AND METHODS

To determine the selectivity of fermentation of the test material in comparison to that of FOS and starch, pH-controlled, stirred batch cultures inoculated with human fecal slurries were used. The bacteriology was carried out using fluores-

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cent in situ hybridization with oligonucleotide probes targeting specific 16S rRNA regions of the microorganisms tested. Short-chain fatty acids (SCFA), as major end products of bacterial fermentation, were analyzed by high-performance liquid chromatography (HPLC).

Materials. All chemicals were purchased from BDH (Poole, Dorset, United Kingdom), except for $MgSO_4 \cdot 7H_2O$ and L-cysteine HCl, which were obtained from Sigma (Poole, Dorset, United Kingdom). Fructo-oligosaccharides (Raftilose P95) were obtained from Orafti (Tienen, Belgium), and starch was from Sigma (Poole, Dorset, United Kingdom). Fresh shaved Hamlin orange albedo (white part of orange peel) was obtained from a Florida commercial citrus juice processor and frozen immediately to −20°C until used.

Extraction of pectic oligosaccharides. Orange albedo was used to extract pectic oligosaccharides. POS were subsequently treated by nanofiltration to remove excess nitrates (14). Orange albedo was mixed with an equal weight of deionized water and then wet milled using a Comitrol model 1700 food processor (Urschel Laboratories, Valparaiso, IN) to a fine paste with an average particle size distribution of less than 0.5 mm. The ground albedo slurry (9 kg) containing 4.5 kg albedo was added to a tank containing 57 liters aqueous HNO_3 (pH 1.5) to give a concentration of 1 g peel/12.5 ml HNO_3, and the was pH adjusted back to 1.5 with concentrated HNO_3. The tank was then agitated for 30 min, and the slurry was pumped into a pasteurizer (MicroThermics HTSL Lab-25, Raleigh, NC) configured with 7.75-mm-inner-diameter tubing in the heat exchangers (400-ml volume heating, 400-ml hold, 400-ml volume cooling). The pasteurizer was adjusted to a heating temperature of 120°C, a back pressure of 310 to 380 kPa (to prevent boiling), and a flow rate of 1,200 ml/min with water prior to pumping the slurry. After switching to the slurry, the steam input temperature had to be increased slightly to give a slurry temperature of 120°C. As set up, the slurry was thus heated to 120°C within 20 s, held at the temperature for 20 s, and then cooled to 14°C within 20 s. Temperature drop in the hold tube assembly was less than 4°C.

The slurry was then pumped through a Pony turbo finisher (Mecat USA, Orlando, FL), a mechanical continuous filtration unit with a 53-μm mesh screen, whereby the insoluble peel residue was separated from the extraction medium. The insoluble peel residue was rinsed using 1 volume of water and agitation for 30 min. The rinse was passed again through the turbo finisher, where the liquid was collected and the insoluble residue was discarded. Isopropyl alcohol (IPA) was then added to the liquid extract to bring the concentration up to 70% IPA, and the solution was stored for 12 h at 5°C to precipitate the pectin. This solution was then pumped through the turbo filter to separate the precipitate, and the alcohol-insoluble precipitate was washed twice more with 2 parts per unit weight of 70% IPA and once with 100% IPA. The alcohol-insoluble fraction was then dried in a vacuum oven.

The alcohol-soluble supernatant was neutralized with NaOH to a pH between 6.5 and 7.5, and the IPA was evaporated off at 35°C under reduced pressure using a Buchi (Brinkman, Westbury, NY) 20-liter rotary evaporator. The aqueous supernatant was centrifuged at 9,639 × *g* to remove a cloudy precipitate that had formed upon adjustment of the pH. The supernatant was poured into a C_{18} solid-phase extraction column (Altex, Harbor City, CA) to separate the polar from the nonpolar fractions. The polar fraction containing POS was lyophilized, and the nonpolar fraction was eluted with ethanol.

Nanofiltration of pectic oligosaccharides. To desalt the polar fraction, continuous diafiltration was performed using 1,000-molecular-weight-cutoff DS-5-DL membranes (Osmonics Desal). The polar fraction (60 g) was dissolved in H_2O (2 liters) to give a total sugar content of 3%. This solution was circulated in the filtration system for 10 min before the initial feed concentrations were measured. Two parallel high-pressure filtration cells were used, with a total membrane area of 81 cm^2. The inlet flow of water into the feed tank was matched to that of the permeate flow rate such that the volumes remained constant. The pressure and temperature were set at 20×10^6 Pa and 60°C, respectively. Permeate flux measurements and retentate and permeate samples were taken every 2 h. The experiment was finished after 6 liters of cumulative permeate was obtained. The retentate containing the orange peel POS was lyophilized. Before and after nanofiltration, the nitrate content of samples was determined by ion chromatography using a Dionex AS14A column, an AG14A guard column, and a 3.5 mM Na_2CO_3–1.0 mM $NaHCO_3$ eluent at a 1.2 ml/min flow rate.

High-performance anion-exchange chromatography (HPAEC). Orange peel POS were separated over a 100-min 25 to 500 mM nonlinear potassium oxalate, pH 6, gradient using a CarboPac PA1 column (4 by 250 mm; Dionex Corp.) and a CarboPac PA guard column on a Dionex 4500i system (18, 19). Polygalacturonic acid was hydrolyzed in an autoclave for 40 min at 125°C in a nitric acid solution. Oligosaccharide standards were isolated by preparative HPLC (17, 20, 21) or obtained from Sigma.

Carbohydrate assays. The total carbohydrate content of POS was determined by the phenolsulfuric acid method (6). The galacturonic acid content was determined by a colorimetric method (8), with some modifications (48). Starch analysis was performed using the Megazyme (Bray, Ireland) total starch analysis kit. This kit utilizes thermostable α-amylase and amyloglucosidase for starch hydrolysis and glucose oxidase/peroxidase for glucose detection.

Monosaccharide analysis. Methanolysis of POS was performed by adding 100 μl methyl acetate and 400 μl 1.5 M HCl in methanol to up to 1-mg samples (dried in 50°C vacuum oven) and heating them at 85°C for 17 h. After the samples had cooled to room temperature, 5 drops of *n*-butanol was added and the samples were evaporated to dryness under a stream of nitrogen. In order to hydrolyze methyl glycosides formed by methanolysis, 0.5 ml of 2 M trifluoroacetic acid was added, and the samples were heated at 121°C for 1 h. The samples were cooled to room temperature and evaporated to dryness, and 0.5 ml of methanol was added and evaporated to dryness three times. Water was added to each sample, and they were filtered (0.25 μm) prior to injection. Inositol was added to each sample prior to methanolysis as an internal standard.

Hydrolysates were analyzed for neutral monosaccharide content by HPAEC-pulsed amperometric detection (PAD) using a Dionex ICS-2500 system that included a CarboPac PA20 column and guard column, an EG 50 eluent generator that produced the isocratic 10 mM KOH mobile phase, a continuously regenerated anion trap column, a GP 50 pump operated at 0.5 ml/min, an ED50 electrochemical detector utilizing the quadruple potential waveform, and an AS50 autosampler with a thermal compartment (30°C column heater). The acidic monosaccharide content was determined with a Dionex DX-500 system, which included a GP50 gradient pump (0.5 ml/min), a CarboPac PA20 column and guard column, an ED40 electrochemical detector (gold working electrode and pH reference electrode), an LC25 chromatography oven (30°C), a PC10 pneumatic controller (postcolumn addition of 500 mM NaOH), and an AS3500 autosampler. The mobile phase consisted of isocratic 10 mM CH_3COONa, 1 mM NaOH eluant for 10 min, and then a linear gradient of 100 to 130 mM CH_3COONa in 100 mM NaOH for the following 20 min.

Fecal batch culture fermentation. Four 280-ml glass fermentor vessels were filled with 180 ml of a prereduced sterile medium containing 2 g/liter peptone water, 2 g/liter yeast extract, 0.1 g/liter NaCl, 0.04 g/liter K_2HPO_4, 0.04 g/liter KH_2PO_4, 0.01 g/liter $MgSO_4 \cdot 7H_2O$, 0.01 g/liter $CaCl_2 \cdot 2H_2O$, 2 g/liter $NaHCO_3$, 0.5 g/liter L-cysteine HCl, 0.5 g/liter bile salts, 4 ml resazurin at 0.05 g/liter, 10 μl vitamin K_1, 2 ml Tween 80, and 10 ml hemin. The medium was adjusted to pH 7.0 and continuously sparged with oxygen-free nitrogen (15 ml/min). A 10% (wt/vol) fecal slurry from three healthy donors was prepared using prereduced 0.1 M phosphate-buffered saline (pH 7.0) and then mixed in a stomacher (model 6041; Seward Scientific, United Kingdom) for 120 s. Each vessel was inoculated with 20 ml of the slurry and 2 g of carbohydrate (POS, fructo-oligosaccharides, orange albedo, or starch). Fecal samples were obtained from one male and two female volunteers with a mean age of 24 years who had not taken antibiotics for at least 6 months prior to providing the sample and who had no history of a gastrointestinal disorder. Fermentors were maintained at 37°C, and duplicate samples were removed after 0, 5, 10, and 24 h for the enumeration of bacteria and SCFA analyses (46, 29). Fermentations were run on three separate occasions with a different donor.

Fluorescent in situ hybridization. Changes in human fecal bacterial populations were assessed by fluorescent in situ hybridization with 16S rRNA probes. These 16S rRNA probes, specific for predominant classes of the gut microflora (bacteroides, bifidobacteria, *Clostridium histolyticum*, *Eubacterium rectale*, and lactobacilli/enterococci), were manufactured and tagged with fluorescent markers. The probes used were Bif164 (26), Bac303 (28), Erec482 (10), His150 (10), and Lab158 (16). The nucleic acid stain 4′6-diamidino-2-phenylindole (DAPI) was used for total bacterial counts. Fermentation samples were diluted and fixed overnight in 4% paraformaldehyde at 4°C in a 1:3 ratio of sample to 4% paraformaldehyde (vol/vol). These cells were then washed with phosphate-buffered saline (0.1 M, pH 7.0), resuspended in 150 μl phosphate-buffered saline plus 150 μl ethanol, and stored at −20°C for a minimum of 1 h. The cell suspension was then added to the hybridization mixture comprised of 4.78 g/liter Tris-HCl (30 mM), 79.69 g/liter NaCl (1.36 M), 15 ml 10% sodium dodecyl sulfate solution (pH 7.2), and 4 M HCl at 15 ml and filtered through a 0.2-μm filter (Millipore, Watford, United Kingdom). Hybridization was carried out at appropriate temperatures for the probes. Subsequently, the hybridization mix was vacuum filtered (Millipore, Watford, United Kingdom), and the filter was mounted on a microscope slide and examined using fluorescence microscopy such that the bacterial groups could be enumerated (36).

Short-chain fatty acid analysis. One-milliliter samples were removed from the batch culture fermentors and centrifuged at 13,000 × *g* for 5 min, and 20 μl was injected onto an HPLC system (LaChrom L7100; Merck, Poole, United

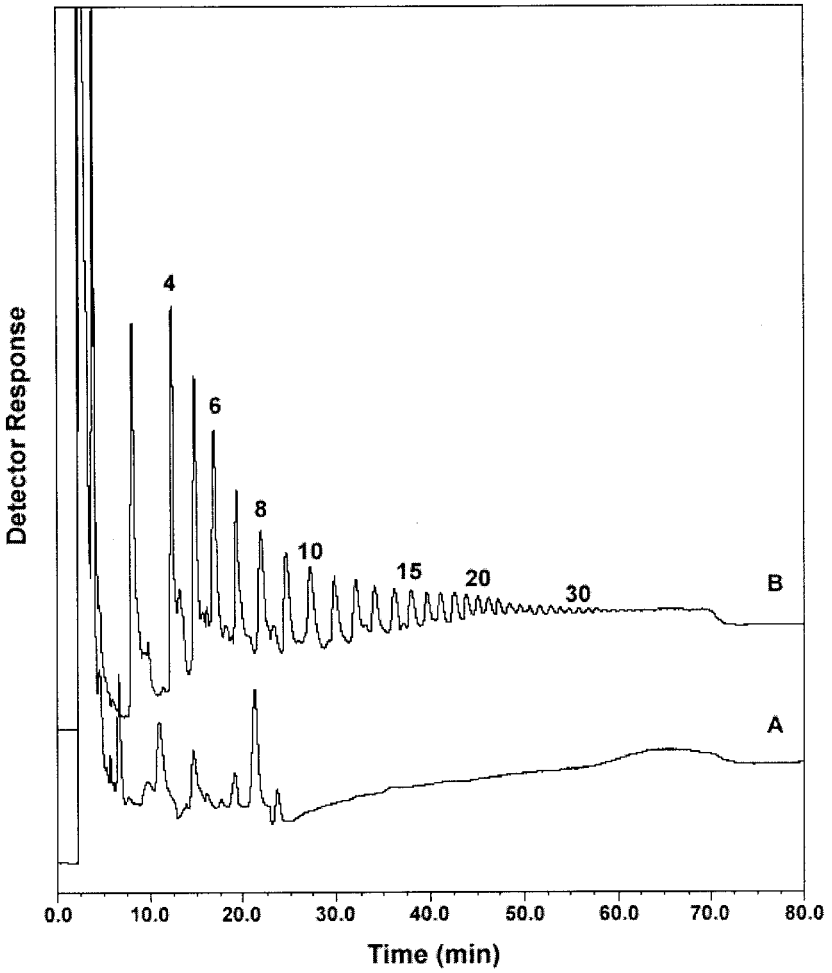

FIG. 1. HPAEC-PAD analysis of POS extracted from orange peel (A) compared to a polygalacturonic acid hydrolysate (B). The degrees of polymerization that corresponded to oligogalacturonic acid standards are indicated above the peaks.

Kingdom) equipped with a refractive index detector and an automatic injector. The column used was an ion-exclusion Aminex HPX-87H column (7.8 by 300 mm; Bio-Rad, Watford, United Kingdom) maintained at 50°C. The eluent was 0.005 mM H_2SO_4 at a flow rate of 0.6 ml/min. Data were acquired using JCL6000 software (Jones Chromatography, Wales, United Kingdom). Quantification of the samples was carried out using calibration curves of acetic, propionic, butyric, and lactic acids in concentrations between 0.5 and 100 mM, and results were expressed in mmol/liter (36). The experiments were repeated in triplicate.

Statistical analysis. Where differences in the treatments were found, a two-sample Student's *t* test was carried out between the samples. The differences were considered significant when P was <0.05.

RESULTS

POS extraction. The pasteurizer extraction of POS from Hamlin orange peel produced 158.5 g of the polar supernatant fraction from 4.542 kg wet peel (976.5 g, dry weight), which was a 16% yield. In order to further purify the polar supernatant fraction and remove salts that could interfere with batch fecal cultures, nanofiltration was performed. Nanofiltration significantly reduced (by five times) the nitrate content to minimal levels and reduced the fraction weight by 50%. Therefore, the final yield of POS from Hamlin orange peel was 8%. An equivalent yield of high-quality pectin was produced (data not shown).

POS composition. Orange peel POS consisted of a series of oligosaccharides (Fig. 1). The POS chromatogram was very similar to the chromatogram for the polar supernatant fraction published previously (see Fig. 2D in reference 21), demonstrating that nanofiltration did not alter the POS oligosaccharide profile. The POS oligosaccharide retention times did not exactly match those of oligogalacturonic acids (Fig. 1), suggesting

TABLE 1. POS monosaccharide composition

Monosaccharide	% of POS
Glucose	48.12
Arabinose	31.19
Galactose	9.59
Xylose	2.44
Rhamnose	2.13
Fucose	0.24
Galacturonic acid	6.29

TABLE 2. Bacterial populations after 0, 5, 10, and 24 h of incubation with different oligosaccharides

Time (h)	Log_{10} cells ml^{-1}[a]											
	Total bacteria				*Bacteroides* spp.				*Bifidobacterium* spp.			
	POS	FOS	OA	STA	POS	FOS	OA	STA	POS	FOS	OA	STA
0	9.42 ± 0.47	9.34 ± 0.20	9.49 ± 0.47	9.59 ± 0.18	8.13 ± 0.09	8.22 ± 0.28	8.10 ± 0.01	8.31 ± 0.19	7.52 ± 0.46	8.05 ± 0.06	7.55 ± 0.41	7.79 ± 0.68
5	9.64 ± 0.45*	9.42 ± 0.17	9.77 ± 0.65	9.93 ± 0.46	8.26 ± 0.2	8.29 ± 0.22	7.81 ± 0.89	8.50 ± 0.17*	8.18 ± 0.34	8.71 ± 0.02*	8.09 ± 0.33	8.37 ± 0.04
10	9.44 ± 0.03	9.55 ± 0.09	9.64 ± 0.05	9.31 ± 0.47	8.36 ± 0.05	8.25 ± 0.14	8.04 ± 0.88	8.50 ± 0.35	8.38 ± 0.73	8.90 ± 0.02*	8.31 ± 0.29	7.94 ± 0.3
24	9.75 ± 0.52	9.25 ± 0.16	9.44 ± 0.22	9.90 ± 0.04	8.22 ± 0.16	8.09 ± 0.26	8.39 ± 0.27	8.43 ± 0.42	8.72 ± 0.35*	8.63 ± 0.23	8.44 ± 0.09	8.20 ± 0.28

[a] Mean bacterial count ± standard error ($n = 3$). *, significantly different from the population at 0 h ($P \leq 0.05$). STA, starch.

that POS had a rhamnogalacturonan structure. When POS was saponified with KOH (pH 11, 30 min) and then neutralized with HCl, the retention times and peak heights of some peaks in Fig. 1 shifted, and a series of minor peaks between 25 and 55 min were observed (data not shown). Retention times of the new peaks that appeared following saponification also did not agree with oligogalacturonic acid retention times. This suggests that some of the galacturonic acid residues in POS were modified, possibly with methyl esters. The exact POS degree of polymerization is unknown. However, since oligosaccharide chain length generally increases with increasing HPAEC-PAD retention times, the orange peel POS chain length range (Fig. 1) was greater than the chain length range of POS I and POS II (22) produced by enzymatic hydrolysis from high-methoxy citrus pectin and low-methoxy apple pectin, respectively (30).

The monosaccharide composition of POS consisted mainly of glucose and arabinose, with lesser amounts of galactose, galacturonic acid, xylose, rhamnose, and fucose (Table 1). POS had a 13.0% (±1.1%) galacturonic acid content and a 56.6% (±1.4%) total carbohydrate content (glucose standard) as determined by the colorimetric assays. The high concentration of glucose present in the POS hydrolysate was due to free glucose in the POS fraction. The glucose content of POS was 18% with and without α-amylase and amyloglucosidase treatment in the starch assay. The presence of free glucose in the unhydrolyzed POS fraction was confirmed by HPAEC-PAD using the CarboPac PA20 column. Free glucose was also detected in the orange albedo (7%) used in this study. The remaining monosaccharide composition was consistent with a rhamnogalacturonan structure containing a 3:1 galacturonic acid/rhamnose ratio. Because this ratio was not 1:1, the POS are probably a combination of rhamnogalacturonan and xylogalacturonan in nature (35, 45). Some of the peaks in Fig. 1 may represent arabinan or arabinogalactan oligosaccharides, which are typical rhamnogalacturonan side chains. It is also possible that small amounts of xyloglucan oligosaccharides could be present based on the POS monosaccharide composition. However, based on how POS was extracted and the fact that there is no evidence for cello-oligosaccharides present in HPAEC-PAD chromatograms, xyloglucan oligosaccharides are probably only minor constituents, if present at all. Further analysis will be performed and subsequently reported to determine the exact POS structure.

Fermentation studies. Changes in human fecal bacterial populations present in the batch cultures are summarized in Table 2. Both POS and FOS produced a significant increase in bifidobacteria after 24 h of fermentation with POS and after 5 and 10 h with FOS. The bacteroides population remained stable for three of the four samples tested, with the exception of starch, where a significant increase was found after 5 h of fermentation. Similarly, *Clostridium histolyticum* levels were maintained with a reduction in numbers after 24 h of fermentation with FOS. Significant increases were seen in *Eubacterium rectale* numbers with POS and FOS, and nonsignificant increases were determined with orange albedo and starch. A rise in lactobacilli/enterococci was also seen, but again, this increase was not significant.

Concentrations of lactic, acetic, and propionic acids were higher at all time points tested with POS as a carbohydrate source (Table 3). POS gave the highest total SCFA of all carbohydrates tested at 24 h. Butyrate production, however, increased after 10 h and peaked at 24 h, coinciding with the highest *Eubacterium rectale* numbers. FOS resulted in significantly increased concentrations of acetic, propionic, and butyric acids after 10 h and lactic acid after 24 h. Fermentation of orange albedo produced increased lactic, acetic, and propionic acids at 5 h, and butyrate concentrations peaked at 10 h and remained elevated up to 24 h, again correlating with *Eubacterium rectale* numbers. After 24 h of fermentation with starch, a significant increase in propionic acid was seen. After 10 h,

TABLE 3. Concentrations of organic acids produced during fermentation of each carbohydrate source at 0, 5, 10, and 24 h at pH 6.8 anaerobic conditions and 37°C[a]

Time (h)	Concn (mM) of organic acid produced during fermentation with:									
	Pectic oligosaccharide					Fructo-oligosaccharide				
	Total	Lactic	Acetic	Propionic	Butyric	Total	Lactic	Acetic	Propionic	Butyric
0	6.24	0.39 ± 0.25	4.37 ± 1.03	0.41 ± 0.51	1.06 ± 0.15	6.43	0.28 ± 0.03	3.94 ± 0.52	0.94 ± 0.9	1.27 ± 0.1
5	26.16	3.96 ± 1.35*	15.19 ± 3.95*	3.91 ± 1.28	2.96 ± 1.60	23.50	0.66 ± 0.54	21.11 ± 1.79*	0.59 ± 0.24	1.14 ± 0.51
10	41.04	6.15 ± 1.08*	17.43 ± 3.20*	6.77 ± 1.41*	10.69 ± 1.42*	48.41	5.71 ± 1.12*	24.07 ± 4.18*	7.93 ± 2.17*	11.18 ± 1.82*
24	73.88	13.10 ± 1.35*	32.76 ± 1.45*	9.23 ± 1.13*	18.09 ± 2.14*	66.61	7.43 ± 1.49*	32.61 ± 4.21*	12.19 ± 2.32*	14.39 ± 2.43*

[a] Values for individual acids are means ± standard deviations. *, result of the paired Student's t test compared to 0 h ($n = 3$).

TABLE 2—*Continued*

Log_{10} cells ml^{-1}[a]												
Clostridium histolyticum group				*Eubacterium rectale*				Lactobacilli/enterococci			
POS	FOS	OA	STA	POS	FOS	OA	STA	POS	FOS	OA	STA
7.49 ± 0.42	7.66 ± .063	7.37 ± 0.14	7.66 ± 0.25	8.27 ± 0.03	8.22 ± 0.04	8.18 ± 0.13	8.37 ± 0.21	6.91 ± 0.27	6.92 ± 0.15	6.70 ± 0.68	6.75 ± 0.61
7.54 ± 0.14	7.88 ± 0.45	7.43 ± 0.22	7.72 ± 0.37	8.54 ± 0.18	8.51 ± 0.02*	8.16 ± 0.23	8.70 ± 0.1	7.18 ± 0.56	7.84 ± 0.18	6.92 ± 0.75	6.95 ± 0.5
7.99 ± 0.97	7.49 ± 0.34	7.24 ± 0.04	8.06 ± 0.96	8.56 ± 0.14	8.63 ± 0.06*	8.23 ± 0.47	8.84 ± 0.35	7.02 ± 0.86	7.29 ± 0.16	6.92 ± 0.91	6.88 ± 0.55
7.48 ± 0.47	7.34 ± 0.11	7.43 ± 0.47	8.15 ± 0.98	8.54 ± 0.02*	8.59 ± 0.02*	8.45 ± 0.3	8.96 ± 0.44	6.84 ± 0.59	7.08 ± 0.26	6.87 ± 0.55	7.56 ± 0.57

butyric acid concentrations increased and remained at a high level until 24 h.

Prebiotic index (PI). A quantitative equation used to help with the analysis of prebiotic fermentation (33) for POS displayed a trend similar to that found in previous research (29), increasing with time (Table 4), as follows:

$$PI_{t_x} = \frac{(\Delta \log_{10} \text{bifidobacteria})_{t_x - t_0}}{(\log_{10} \text{total counts})_{t_x}} + \frac{(\Delta \log_{10} \text{lactobacilli})_{t_x - t_0}}{(\log_{10} \text{total counts})_{t_x}} - \frac{(\Delta \log_{10} \text{bacteroides})_{t_x - t_0}}{(\log_{10} \text{total counts})_{t_x}} - \frac{(\Delta \log_{10} \text{clostridia})_{t_x - t_0}}{(\log_{10} \text{total counts})_{t_x}}$$

FOS displayed a similar trend but achieved higher values. OA displayed an initially high PI score, but this decreased with time. The PI for starch was found to decrease after 10 h, possibly due to increased levels of bacteroides and clostridia at this time.

DISCUSSION

Batch culture experiments were carried out with POS and OA, which are by-product streams of orange juice manufacturing, and compared for their prebiotic potential against FOS and starch as control substrates. While the orange peel POS and OA contained free glucose, this carbohydrate is not known to have prebiotic properties. Therefore, the POS derived from orange peel were responsible for the elevated PI values. Previously, enzymatically generated POS have been reported to have potential as prebiotic substrates (29), with elevated PI relative to those of their parent pectins. The selectivity was not, however, as marked as that seen with FOS, probably due to the maintenance of bacteroides and clostridium populations throughout the fermentation. Pectin substrates have been shown to maintain the levels of all bacteria in previous fermentation experiments (7). Due to variation in fecal donors, the PI values obtained for enzymatically generated POS (29) and the POS PI values obtained here cannot be compared directly. However, it is likely that the orange peel POS PI would continue to increase after 24 h, as was observed for enzymatically generated POS (29). Orange peel POS also increased the bifidobacterial population later in the fermentation than did FOS, suggesting that the orange peel POS produced a more sustained prebiotic fermentation. A blend of POS and FOS might result in elevated bifidobacterial numbers for a prolonged period of time (9).

The use of a tubular heat exchanger for the continuous extraction of sugar beet pectin was reported previously (47). This is the first report of methods to produce both pectin and POS from orange peel. While the yield of POS was 8%, our preliminary estimate is that it would not be more expensive to produce commercially than pectin. POS is a significant component of the supernatant remaining after pectin precipitation. This supernatant is a waste material with a high biological oxygen demand that is very expensive to dispose of during pectin manufacturing. Therefore, POS is potentially a coproduct that could lower the cost of pectin manufacturing.

Eubacterium rectale numbers significantly increased with the oligosaccharides tested. Dietary fiber in the form of fruit pectin has previously been shown to increase eubacterial numbers (7, 38). In addition, pectins with different degrees of esterification result in differing butyrate concentrations (5). This physiologically important group of bacteria is known to produce relatively large amounts of butyrate (38, 1, 24). Butyrate production is believed to be beneficial to health (4), with touted roles in the prevention of ulcerative colitis (3, 39, 41) and colon cancer (15, 34).

In conclusion, we have shown that POS can be used effectively as a prebiotic because of the increase in bifidobacteria and *Eubacterium rectale* numbers with the subsequent increase in butyrate concentrations, giving an added possible health benefit.

TABLE 3—*Continued*

Concn (mM) of organic acid produced during fermentation with:									
Orange albedo					Starch				
Total	Lactic	Acetic	Propionic	Butyric	Total	Lactic	Acetic	Propionic	Butyric
7.16	0.27 ± 0.05	4.95 ± 0.51	0.85 ± 0.26	1.09 ± 0.24	7.16	0.29 ± 0.03	4.97 ± 0.77	0.72 ± 0.24	1.19 ± 0.23
14.99	3.28 ± 0.57	5.91 ± 1.14*	3.21 ± 0.21*	2.59 ± 1.51	8.95	0.31 ± 0.16	5.30 ± 1.62	1.56 ± 0.49	1.79 ± 1.21
36.48	3.34 ± 0.05	15.10 ± 3.87*	7.02 ± 0.25*	11.02 ± 3.16*	13.98	0.62 ± 0.17	5.71 ± 0.62	3.54 ± 0.67	4.20 ± 0.49
45.27	3.58 ± 1.42	21.85 ± 3.64*	8.56 ± 1.69*	11.28 ± 2.61*	19.52	0.68 ± 0.12	9.11 ± 0.81*	3.56 ± 1.23*	6.17 ± 1.38*

TABLE 4. PI of POS, FOS, OA, and starch after 5, 10, and 24 h of anaerobic fermentation at pH 6.8 and 37°C

Oligosaccharide	PI at:		
	5 h	10 h	24 h
POS	3.51	5.37	7.65
FOS	9.98	6.32	7.84
OA	2.32	4.9	8.98
Starch	2.21	3.15	4.17

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